REVIEW 10.1111/j.1469-0691.2008.02692.x

Impact of space, time and complex environments on microbial communities

A. Ramette[1], J. M. Tiedje[2] and A. Boetius[1]

1) *Max Planck Institute for Marine Microbiology, Bremen, Germany and* 2) *Michigan State University, East Lansing, MI, USA*

Abstract

The unparalleled accumulation of biological and contextual data is currently revolutionizing the way environmental microbiologists address ecological questions. Here, we briefly review the likely causes that may explain this remarkable scientific revolution and present a synthesized view about how to describe microbial communities in their complex environmental context.

Keywords: Ecology, diversity, modelling, theory, multivariate

Clin Microbiol Infect 2009; **15** (Suppl. 1): 60–62

Corresponding author and reprint requests: A. Ramette, Microbial Habitat Group, Max Planck Institute for Marine Microbiology, Bremen, Germany
E-mail: aramette@mpi-bremen.de

Environmental microbiology is currently undergoing a revolution owing to the increasing accumulation of biological information and of contextual environmental parameters [1–4]. This new era may well be described as a microbial ecologist's dream. Key ecological questions such as the extent of variation of microbial diversity and abundance with respect to spatial, temporal or environmental parameters may start to be addressed. Here, we briefly review the likely causes for this dramatic advance in discovery and present a synthesized view about how to describe microbial communities in their complex environmental context. Examples from our own work are presented to illustrate possible combinations of methods, strategies and approaches that could lead to new discoveries.

Microbial communities thriving in different habitats in soil or marine environments may be studied via a combination of high-throughput molecular techniques and powerful multivariate analyses (Fig. 1). Microbial diversity can be routinely inferred using variations in molecular markers (e.g. 16S rRNA genes, protein-coding genes) and phenotypic characteristics. The abundance of organisms can be quantified using plate counting (for the culturable fractions) or by microscopic observations (e.g. fluorescent *in situ* hybridization). The diversity among and between communities and populations can thus be determined using phylogenetic reconstruction. Environmental heterogeneity can be measured by collecting contextual information about the microbial habitat from which the organisms were isolated. Because most microbial ecology studies are of an exploratory nature (i.e. one cannot tell *a priori* what the main environmental factors are), multivariate analyses may be used to reduce the dimensionality of those additional datasets [3]. In natural ecosystems, complexity and multidimensionality are common features: multiple factors, variables and parameters are generally collected and stored in specialized databases. There is thus a need to quantify the amount of biological variation that can be explained by environmental, temporal and spatial factors in order to establish the strength of our current understanding and predictive abilities about changes in the microbial world. This ecosystem approach may shed light on the predictability of natural processes governed by microbial populations. A summary of the overall strategy that combines microbial data with environmental, spatial and temporal parameters is presented (Fig. 1).

The current revolution in microbial ecology is taking place because the primary materials (i.e. DNA and protein sequences) that microbiologists use to study large-scale diversity patterns are being accumulated at an exponential rate, and because of new molecular techniques (e.g. pyrosequencing, whole genome amplification, and metagenomics), which are speeding up the process even further [5]. In-depth estimation of microbial diversity will probably be soon complemented by assessing numerous samples in parallel, a process that is so far rather limited. It can be assumed that in the near future a full understanding of the depth and breadth of microbial diversity will be possible, thus providing new insights into the central role of microbes on earth.

Refinement of existing molecular techniques may also lead to significant improvements in the description of diversity patterns. For instance, in multilocus sequence typing

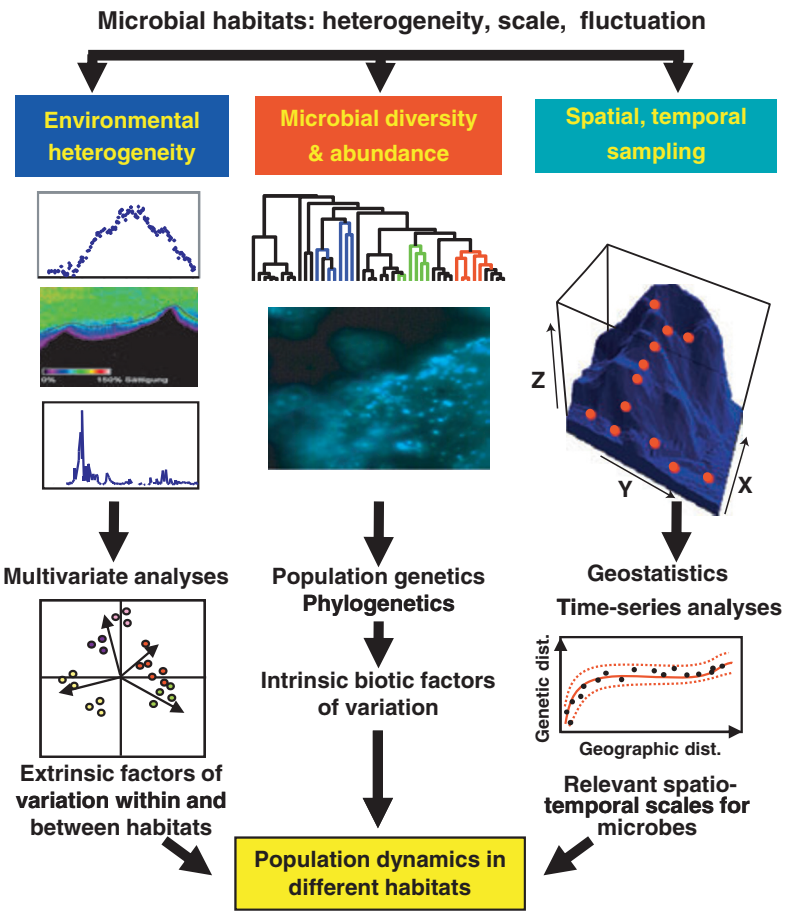

Fig. 1. Synthetic scheme to combine microbial diversity and abundance with environmental, spatial and temporal parameters in a coherent framework.

approaches, seven to ten loci are typically used to compare evolution across microbial strains. We showed that not all markers yielded meaningful results when compared with the phylogenetic information deduced from genomic data [6]. A more accurate phylogeny of the *Escherichia coli* group was obtained on the basis of just three genes, in comparison with the concatenated alignment of eight genes that are commonly employed for phylogenetic purposes. Those results were reproducible within *Salmonella*, *Burkholderia* and *Shewanella*, suggesting a broad applicability for those analyses.

Despite huge progress in obtaining sequence information, advances in the isolation and cultivation of microbes remain central to a better understanding of the coupling of diversity, function and metabolic potential of specific strains from the environment. For instance, the diversity of *Burkholderia cepacia* isolates was investigated at the community, species and intraspecific level by using a combination of molecular and culture-dependent techniques [7]. Efficient strategies to detect, isolate and screen large numbers of *B. cepacia* isolates from soil and rhizosphere samples were used to offer a fast and reliable diagnostic assay for members of this bacterial complex that colonize samples as different as soil, water, sediment, or human lungs. The combination of molecular data with advanced ecological modelling tools also proved valuable. Variations in community composition, abundance and diversity were determined as functions of environmental and spatial parameters for various rhizosphere samples [8]. A powerful spatial modelling approach was used to examine all spatial scales that significantly structured the microbial community and to relate spatial scales with environmental heterogeneity [9]. It was then possible to determine the amount of biological variation that could be explained by different spatial scales and environmental fluctuations of contextual physicochemical parameters at different taxonomic levels. Interestingly, the intraspecific diversity showed significant variation as a function of nearly all scales present in the sampling strategy. This may indicate very complex structuring of both space (i.e. historical factors) and contemporary environmental parameters at the genotype level in microbial populations [8]. Microbial ecologists have traditionally favoured the environmental control over the historical (spatial distance) hypothesis to explain microbial patterns in the environment [1,4,8]. Determining the effects of spatial structure in biological data is a recurring topic in traditional

landscape ecology, and microbiologists should take advantage of the numerous existing examples to apply those approaches to their own studies. Statistical tools, in this respect, may be of great help in separating the respective effects of pure spatial and pure environmental variation [3]. Also, their covariation, i.e. how much spatial structuring indirectly affects biotic variables through environmental structuring, can be quantified [10].

Marine coastal sediments are examples of highly productive ecosystems that play important roles in carbon and nitrogen recycling, but whose microbial communities are still poorly understood. Not only do sediments cover a large area of the earth's surface, but they are also highly colonized by microbial communities. We recently applied the 454 massive parallel tag sequencing technique to sandy sediments from the North Sea island Sylt through a collaboration with the International Census of Marine Microbes (ICoMM; http://icomm.mbl.edu/). The objectives were to obtain a basic understanding of the depth of the microbial diversity in coastal sediments and to identify the main environmental drivers that may structure sand-associated communities. The 454 massively parallel tag sequencing strategy has been shown to be able to detect the rare biosphere that generally escapes traditional molecular approaches [11]. Multivariate analyses, when applied to massive parallel tag sequencing-generated data, could extract the main amounts of variation from highly dimensional tables, despite the huge dataset size of more than 160 000 sequence tags. The results suggest that a broad range of sequence tags are present in Sylt sands, consistent with an extensive diversity and high turnover of community diversity in such dynamic ecosystems (A Gobet, A Ramette, ML Sogin and A Boetius, unpublished).

Although the tendency to work with larger datasets is becoming obvious, a pitfall would be to blindly embark on generating more data without clear objectives. The accumulation of data should ideally be accompanied by an accumulation of knowledge. Several authors have already warned us against this lack of strategy [2]. The development of sound theories that are falsifiable is needed to provide the necessary scheme to advance science into a coherent framework. To reach this goal, environmental microbiologists have to face the big challenge of understanding complexity and diversity by constantly updating their approaches, theories and methods.

In conclusion, it is now possible to assess the impact of space, time and complex environments on microbial communities and to quantify interactions among factors. This new possibility has been made possible by a conjoint revolution in data acquisition, storage and processing. The next challenge may be the generation of microbial diversity theories that will allow further comparisons with those of macro-organisms or that can be tested across various ecosystems. For instance, recent developments in the study of microbial biogeography [1,4,12] may be seen as a prelude to a more dramatic revolution in the understanding of changes in microbial communities in complex environments.

Transparency Declaration

All authors declare no conflicts of interests.

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